

Experiment #5: Thin-Layer Chromatography: Identification of Over-the-Counter Analgesics

CHEM 213 – Fall 2008

Thin-layer chromatography (TLC) is a fast and simple method that can be used to identify organic molecules, verify the number of components in a mixture, or to monitor the progress of a reaction. In this experiment, you will be identifying the active ingredients (called analgesics) in over-the-counter painkillers, such as Tylenol, Motrin, Excedrin and Anacin. This will be accomplished by synthesizing the drugs and using them as the standards (controls) for the TLC analysis.

In order to save time, you will only synthesize one pure analgesic then work in collaboration with two classmates to complete the identification. The instructor will assign who will be responsible for synthesizing each of the following three analgesics: aspirin, acetaminophen or phenacetin. Follow the scale indicated for your particular synthesis. After the syntheses are complete, you will be performing your own TLC analysis. TLC is a microscale procedure.

Objectives:

1. Synthesize and purify phenacetin, aspirin or acetaminophen using the procedures below.
2. Determine the R_f values for the four standards; phenacetin, acetaminophen, aspirin and caffeine (provided).
3. Determine the number and identity of the components in the four unknown OTC analgesics.

Helpful Hints:

1. Do not spot too heavily. The spots will run and give poor results.
2. Make sure your spotting capillaries and developing jar are free from contaminants.
3. Keep track of your TLC plates. Only label them with pencil, as the ink is organic and will run along with your spots. Do not touch the absorbent side of the plate. The oils from your fingers will also give spots and confuse your results.
4. Standard solutions must be very low in concentration, i.e. 1% solution in ethanol. These solutions can be shared with your classmates. However, be consistent and always use the same standard when comparing your values.
5. A common solution will be provided as the TLC solvent. It is a mixture of 99.5 % ethyl acetate/0.5% acetic acid. Do not make this solution yourself. Please use the one provided in the front of the lab.
6. The TLC plates are quite expensive, so please use them sparingly. The instructor will provide them.
7. The unknown over-the-counter pain medications will be 1% solutions in ethanol as well. These test vials will also be provided for you.

Reading:

Technique 15.1-15.8 and the synthetic procedures on the following page.

Prelab* Questions:

1. A TLC of two substances was eluted using 2-propanol, both substances had an $R_f = 1$. Are these two substances identical? Explain. If not, what modifications would you make to the experiment to draw a more accurate conclusion.
2. The R_f value for compound A is 0.34 when hexanes is used as the eluting solvent and 0.44 for diethyl ether. Compound B has an R_f of 0.42 in hexanes and 0.60 in ethyl ether. Which would be the better solvent of choice for separating the mixture? Briefly explain.
3. Using online resources, determine what are the major components in the following OTC analgesics Tylenol, Motrin, Excedrin and Anacin?

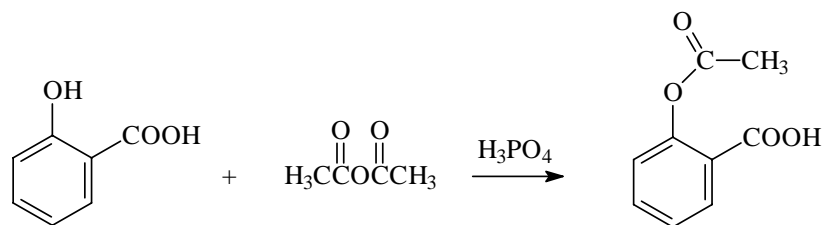
* This lab is the first involving a chemical reaction. Your Pre-Lab MUST include the balanced chemical equation and chemical data table for the one analgesic synthesis that you are assigned by your instructor. The data table should follow the format given in the Notebook Primer (online).

Post Lab Questions:

1. Assign the four unknown analgesics to the four OTC analgesics in Prelab question 3.
2. Explain the order of elution of the acetaminophen, aspirin and phenacetin based on their structures.
3. In this experiment you used 99.5/0.5 ethyl acetate/acetic acid as your elution solvent. Suppose you changed the ratio to 90/10 ethyl acetate/acetic acid, how would the R_f values of your components change? Would they increase or decrease? Briefly explain. (Note: the polarity of acetic acid is similar to that of water)

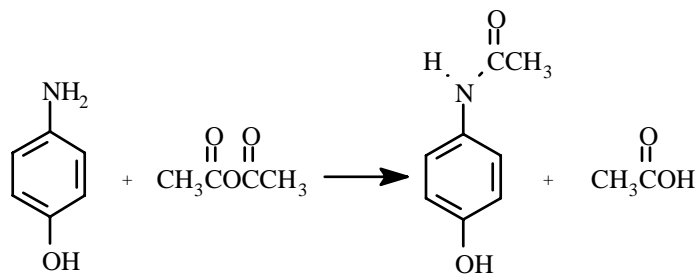
Synthetic Procedures:

Aspirin¹



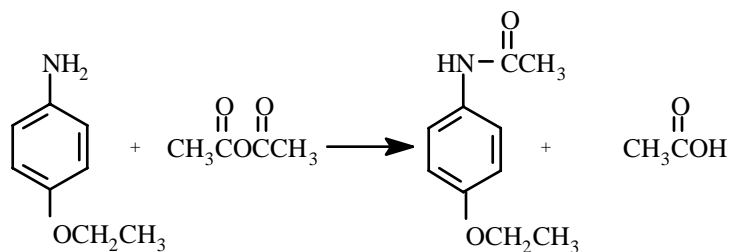
Heat water in a beaker using a hot plate. To a large test tube, add 1 g of salicylic acid, a boiling chip, and 8 small drop of 85% H_3PO_4 . Add 2 mL of acetic anhydride, which should be used to wash the reactants to the bottom of the tube. Mix the reactants thoroughly with a glass stirring rod, and then heat the reaction tube in the beaker of hot water at $\sim 90^\circ\text{C}$ for 5 min. **Cautiously** add 1.5 mL of d H_2O drop wise to the reaction mixture to decompose excess acetic anhydride. This will be an exothermic reaction. When the reaction is over, add 2 mL more water and allow the tube to cool slowly to room temperature. Allow the solution to sit for 10 min. If crystallization does not occur during the cooling process, add a seed crystal or scratch the inside of the tube with a glass stirring rod. Cool the tube in ice until crystallization is complete, and then remove the crystals using vacuum filtration. Wash the crystals with a very small quantity of ice water. Boiling water hydrolyzes aspirin, but the reaction is not rapid; therefore, the product may be quickly recrystallized from a small quantity of very hot water. Put the product onto a piece of filter paper and squeeze the crystals between sheets of filter paper to absorb excess water and let it dry thoroughly in air for at least 24 hours.

Acetaminophen²



Weigh approximately 0.5 g of 4-aminophenol in a 5 mL round bottom flask equipped with a boiling chip and an air condenser. Add 1.5 mL of water and 0.6 mL of acetic anhydride ($d=1.08 \text{ g/mL}$). Carefully reflux the mixture on a sand bath ($\text{temp} = 115^\circ\text{C}$) with occasional swirling. After the solid has dissolved, (it may dissolve, precipitate and redissolve) heat the mixture for an additional 10 min. When the reaction has cooled to RT, place it on ice to induce crystallization. After cooling for 15-20 min, collect the crystals by vacuum filtration, and wash them with 3 x 2 mL portions of ice water. Recrystallize the crystals from a 50/50 mixture of methanol/water. Allow the crystals to dry and record a melting point.

Phenacetin³



Weigh out 1.0 mL of 4-ethoxyaniline and transfer it to a 50 mL Erlenmeyer flask. Add 20 mL of distilled water, followed by 0.6 mL concentrated HCl. Swirl the mixture to dissolve the 4-ethoxyaniline. Treat the solution with decolorizing carbon and gravity filter. Weigh out 1.1 g sodium acetate trihydrate, place it in a 25 mL Erlenmeyer flask and dissolved the salt in 5.0 mL distilled water. Set the solution aside. Transfer the acidic solution of 4-ethoxyaniline to a 50 mL Erlenmeyer flask, add 0.85 mL acetic anhydride ($d=1.08$ g/mL) to the aqueous solution, swirl once and then add the sodium acetate solution all at once and swirl vigorously. The reaction is very rapid and product precipitates from the solution almost immediately. Swirl the flask for 10 min and then cool it in an ice bath for 10-15 min. Collect the solid product by vacuum filtration and wash with small portions of cold water. Recrystallize the material from ethanol or from ethanol-water. Allow the crystals to dry and record a melting point.

Caffeine

You will also use caffeine as a standard for identification of the ingredients in the over-the-counter medications as caffeine is a common ingredient to avoid drowsiness. The instructor will provide a 1% standard solution of caffeine in ethanol.

References:

1. Adapted from Williamson, K.L. Macroscale and Microscale Organic Experiments, 4th Edition. Houghton Mifflin, 2003; pp. 503-506.
2. Pavia, Donald L.; Lampman, Gary E., Kriz, George S., Engel, Randall G. Organic Laboratory Techniques. Saunders College Publishing, 1990; pp. 64-66.
3. Durst, H. DuPont, Gokel, George W. Experimental Organic Chemistry. McGraw-Hill, Inc. 1987; pp. 369-372.